

Certificate of Analysis

RSK3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-462, 14-462-K, 14-462M

Parent Lot # D7AN006U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged recombinant full-length, human RSK3, expressed in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Activated using MAP Kinase 2 (cat# 14-173) and PDK1 (cat# 14-452) and repurified using glutathione-agarose, heparin Sepharose and Ni²⁺/NTA-agarose. Purified to 27% by SDS-PAGE and Coomassie blue staining. MW = 87kDa.

Specific Activity (Parent lot# D7AN006U): 243U/mg, where one unit of RSK3, active activity is defined as 1nmol phosphate incorporated into 30μM (KKKNRTLSSVA) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 1.688mg/ml of enzyme in 50mM Tris/HCl pH7.5, 0.1mM EGTA, 150mM NaCl, 0.03% Brij-35, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol, 270mM sucrose. Frozen solution.

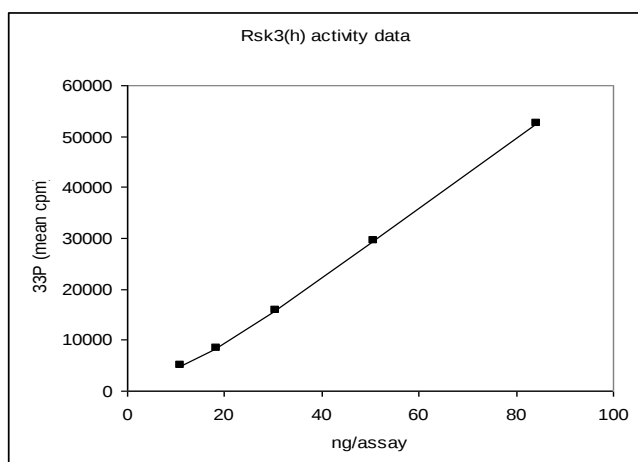
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

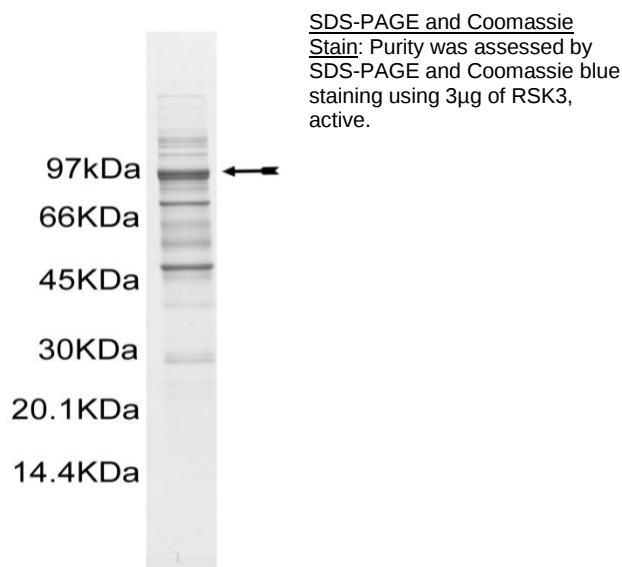
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 11–84ng of this lot of enzyme phosphorylated 30μM (KKKNRTLSSVA) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as RSK3 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS pH7.0, 1mM EDTA.
2. **(KKKNRTLVA):** Use at a final assay concentration of 30 μ M. Prepare a 300 μ M stock and add 2.5 μ l of stock per assay point.
3. **RSK3, active:** Dilute with 20mM MOPS pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 11–84ng per assay point.
4. **[γ -³³P]ATP:** 2.5x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(KKKNRTLVA)**.
3. Add **2.5 μ l (11–84ng) RSK3, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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RSK3 Sequence Information

<u>Protein</u>	human RSK3
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of human RSK3
<u>Accession number</u>	GenBank XM_004469

Recombinant RSK3 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MDLSMKKFAV RFFSVYLRR KRSKSSSL
61 RLEEEGVVKE IDISHHVKEG FEKADPSQFE LLKVLGQGSY GKVFLVRKVK GSDAGQLYAM
121 KVLKKATLKV RDRVRSKMER DILAEVNHPF IVKLHYAFQT EGKLYLILDF LRGGDLFTRL
181 SKEVMFTEED VKFYLAELAL ALDHLHSLGI IYRDLKPENI LLDEEGHIKI TDFGLSKEAI
241 DHDKRAYSFC GTIEYMAPEV VNRRGHTQSA DWWSFGVLMF EMLTGSLPFQ GKDRKETMAL
301 ILKAKLGMPQ FLSGEAQSL RALFKRNP CN RL GAGIDGVE EIKRHPFFVT IDWNTLYRKE
361 IKPPFKPAVG RPEDTFHFDP EFTARTPTDS PGVPPSANAH HLFGRGFSFVA SSLIQEPSQQ
421 DLHKVPVHPI VQQLHGNNIH FTDGYEIKED IGVGSYSVCK RCVHKATDTE YAVKIIDKSK
481 RDPSEEIEIL LRYGQHPNII TLKDVYDDGK FVYLMELMR GGELLDRI LR QRYFSEREAS
541 DVLCTITKTM DYLSHQGVVH RDLKPSNILE RDESGSPESI RVCDFGFAKQ LRAGNGLLMT
601 PCYTANFVAP EVLKRQGYDA ACDIWSLGIL LYTMLAGFTP FANGPDDTPE EILARIGSGK
661 YALSGGNWDS ISDAAKDVVS KMLHVDPHQR LTAMQVLKHP WVVNREYLS P NQLSRQDVHL
721 VKGAMAATYF ALNRT PQAPR LEPVLSSNLA QRRGMKRLTS TRL

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Recombinant RSK3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tccaacgac cgaaaacctg
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361 aaggtcctta agaaagccac ctaaaagttt cgggaccgag tgagatcgaa gatggagaga
421 gacatcttgg cagaagtga tcaccccttc attgtgaagc ttcattatgc ctttcagacg
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1621 gacgtcctgt gcaccatcac caagaccatg gactacctcc attcccaggg ggtgtgtcat
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1861 gcgtgtgaca tctggagttt ggggatcctg ttgtacacca tgctggcagg atttaccct
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2281 acgcggctgt ag

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